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IMMUNE REACTIONS IN RABBITS INJECTED WITH MICROCOCCI FROM ACUTE POLIOMYELITIS*

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In previous communications[1] one of us described a peculiar micrococcus obtained in cultures of the brain and spinal cord in acute poliomyelitis. The exact significance of the coccus has not been determined, but the results of cultures and experiments suggest that it may be of some importance. It has been found that opsonin apparently specific for this micrococcus occurs in the blood of persons recovering from poliomyelitis.[2] In order to arrive at a clearer understanding of the immunity reactions it seemed advisable to study the serum of rabbits, which were immunized against different strains of the coccus, with especial reference to the opsonins, complement fixing bodies, and agglutinins.

The bacteria were grown in ascites dextrose broth for 18 to 24 hours, then removed by centrifugation, washed, and suspended in sterile salt solution in sufficient amount to make a faintly cloudy, smooth suspension. Rabbits, 3 to 6 months old, were inoculated intravenously at 3 or 4 day intervals with increasing amounts, 0.25 - 2 c.c., of this suspension. After the rabbits had received several injections at short intervals and antibodies had accumulated in the blood, subcutaneous injections of 2 c.c. of the suspension were given weekly to maintain the antibody-content. Using similar methods, other rabbits were injected with strains of pneumococcus, of streptococcus viridans, of hemolytic streptococcus, and of B. typhosus, and their sera studied also. A moderate amount of antibody usually appeared after the 4th or 5th inoculation, and this increased decidedly after the 7th or 8th. When 0.02 c.c. of the rabbit serum gave complete inhibition of hemolysis with $\frac{1}{120}$ of the anticomplementary unit of the corresponding antigen, the rabbit was then bled from the heart, the serum heated to 56 C. for 30 minutes, and stored in the refrigerator.

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[1] Mathers: Jour. Am. Med. Assn., 1916, 67, p. 1019; Jour. Infect. Dis., 1917, 20, p. 113.

[2] Mathers and Tunnicliff: Am. Med. Assn., 1916, 67, p. 1935.

COMPLEMENT FIXATION

Antisheep rabbit system was used in one-tenth the volume of the original Wassermann test. Two series of tests were made with each immune serum—varying the amount of antigen in one instance and the amount of serum in the other. As antigens similar suspensions of bacteria as for inoculation were used, with the exception that now they were heated to 56 C. for 30 minutes. The anticomplementary unit of each antigen was determined. In the tests in which the serum remained constant, the amount of antigen was varied from one-fourth to one one hundred and twentieth of the anticomplementary unit. When the serum was varied a constant amount of antigen representing one-fourth of the anticomplimentary unit was used.

In the first experiments both heated and unheated serum was used, but the unheated serum was so frequently anticomplementary that its use was abandoned. When the antigen was varied, 0.02 c.c. of the serum was used. When the serum was varied, 0.02 c.c. was the first dilution and 9 subsequent dilutions were included. Fresh guinea-pig serum, 0.1 c.c. of a 1:10 dilution (2 units), was used as complement. Since it has been found that the serum of normal rabbits may give a positive Wassermann reaction, it seemed best to study the serum of each rabbit before inoculation. Accordingly several cubic centimeters of blood were drawn from the marginal ear vein of each rabbit and in every instance the serum was tested with the organism intended for antigen, with syphilitic liver extract and ascites dextrose broth. In no instance did the serum have any binding power. The Wassermann and ascites dextrose broth tests were repeated again after the animals were immunized; in no instance were positive results obtained.

Serum, antigen, and complement were incubated for 1 hour at 37 C.; then 0.1 c.c. of a 5% suspension of washed sheep corpuscles, and two units of antisheep amboceptor were added to each tube, the whole incubated at 37 C. for from 15 to 30 minutes according to the rapidity of the hemolysis in the controls. The customary controls (antigen, serum, and hemolytic), were carried through in each test.

So far observations have been made on the sera of 12 rabbits. Eight of these were injected with different strains of cocci from poliomyelitic material, one each with a pneumococcus, one with a hemolytic streptococcus, one with a Streptococcus viridans, and one with B. typhosus. The serum of a normal rabbit was included in each test as control. The results of the complement fixation tests using the two methods have been recorded in Tables 1 and 2.

TABLE 1.

Complement Fixation with the Serum of Rabbits Immunized with Micrococci from Poliomyelitis

Serum of Rabbits, Injected with Organisms as follows:	Antigens																
												Controls					
	Tonsil, Poliomyelitis	Poliomyelitis 1	Poliomyelitis 3	Poliomyelitis 4	Poliomyelitis 5	Poliomyelitis 6	Poliomyelitis 7	Poliomyelitis 8	Poliomyelitis 9	Poliomyelitis 10	Poliomyelitis 11	Pneumococcus	Hemolytic Streptococcus	Streptococcus Viridans	B. Typhosus	Luetic Liver	Ascitic Dextrose Broth
Tonsil, poliomyelitis........	++++	+++	++++	+++	++++	+++	+++	++++	++++	++++	+++	—	+++	++	—	—	—
Poliomyelitis 7.............	+++	—	+	+++	++	+	++++	+	+	++	+++	—	+++	++	—	—	—
Poliomyelitis 7.............	++	+++	+++	++	+++	++++	++++	—	+	+++	+	+	+++	—	—	—	—
Poliomyelitis 10............	++++	+++	++	++	—	+	++++	++++	++	++++	—	—	—	—	—	—	—
Poliomyelitis 6.............	++++	+	—	++	++++	++++	++++	++++	++	++++	—	—	+	+	—	—	—
Poliomyelitis 8.............	++++	+	—	+++	++++	+	++++	++++	++++	++++	—	—	++++	+	—	—	—
Poliomyelitis 11............	++++	++++	—	++	++++	++	++++	++++	++++	++	++++	—	++	+	—	—	—
Poliomyelitis 1.............	+++	++++	—	+++	++++	++	++	+++	++++	++++	++++	—	—	—	—	—	—
Controls																	
Pneumococcus...........	++	++	++	++	++	+	+	++	++	++	+++	++++	++	—	—	—	—
Hemolytic streptococcus	—	—	—	—	+++	—	—	++	++	—	++	++	++++	+	—	—	—
Streptococcus viridans..	—	—	—	—	—	—	—	—	—	—	—	+	—	++++	—	—	—
B. typhosus.............	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Normal rabbit serum....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

— = Complete hemolysis.
+ = Inhibition of hemolysis in 1/4–1/8 of anticomplementary unit.
++ = Inhibition of hemolysis in 1/16 of anticomplementary unit.
+++ = Inhibition of hemolysis in 1/32–1/64 of anticomplementary unit.
++++ = Inhibition of hemolysis in 1/128, etc., of anticomplementary unit.

TABLE 2.

COMPLEMENT FIXATION WITH THE SERUM OF RABBITS IMMUNIZED WITH MICROCOCCI FROM POLIOMYELITIS

Serum of Rabbits, Injected with Organisms as follows:	Antigens																
												Controls					
	Tonsil, Poliomyelitis	Poliomyelitis 1	Poliomyelitis 3	Poliomyelitis 4	Poliomyelitis 5	Poliomyelitis 6	Poliomyelitis 7	Poliomyelitis 8	Poliomyelitis 9	Poliomyelitis 10	Poliomyelitis 11	Pneumococcus	Hemolytic Streptococcus	Streptococcus Viridans	B. Typhosus	Luetic Liver	Ascitic Dextrose Broth
Tonsil, poliomyelitis	.0001	.01	.0001	.0012	.00003	.00125	.0006	.0025	.0003	.0025	.01	.005	01	.005	0	0	0
Poliomyelitis 7	.0003	.005	.005	.05	.01	.005	.0003	.00125	.00125	.0025	.005	.0025	.01	.01	0	0	0
Poliomyelitis 7	.0006	.005	.00125	.0003	.00003	.01	.00008	.0015	.0006	.00015	.02	.0025	.005	.01	0	0	0
Poliomyelitis 10	0	.005	.0003	.00125	.005	.0006	.01	.00125	.0025	.00125	.01	.0025	0	0	0	0	0
Poliomyelitis 6	.0025	.01	.005	.005	.005	.0006	.005	.01	.0012	.01	.01	.005	.05	.01	0	0	0
Poliomyelitis 8	.005	.02	.0025	.0006	.005	.0006	.0025	.0025	.005	.0025	.005	.005	.005	.01	0	0	0
Poliomyelitis 11	.0003	0	.005	.005	.003	.0006	.005	.0025	.005	.005	.0025	.005	0	0	0	0	0
Poliomyelitis 1	.0003	.0003	.005	0	0	.0006	.005	.00015	.00125	.005	.0003	.02	0	0	0	0	0
Controls																	
Pneumococcus	0	.01	0	0	0	.005	0	0	0	0	.01	.0003	.01	0	0	0	0
Hemolytic streptococcus 217	0	0	0	.0012	0	.0025	0	0	.005	0	0	.01	.0006	0	0	0	0
B. typhosus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normal rabbit	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The figures in the table represent the lowest dilution of serum with which there was complete complement fixation.

The noteworthy points might be briefly reviewed. Antibodies for the homologous poliomyelitic coccus usually developed a short time before the group antibodies, but the general tendency of the complement-fixing substances was toward specificness for the group of poliomyelitis cocci. The serum of a rabbit injected with a poliomyelitic strain would bind complement with the majority of the poliomyelitis antigens but not with the control antigens, and vice versa. However, in some instances pneumococcus and streptococcus antigens gave positive reactions with the sera, but usually in lower dilutions than with the poliomyelitic antigens. Occasionally a serum with high fixing power with most of the strains would not contain any fixing substances for some one strain. Such discrepancies may be due to unknown physicochemical conditions that are unavoidable. The Tables show that on the whole the group specificity is marked.

OPSONINS

The methods used were similar to those described in connection with the opsonic reaction in human cases.[3] Normal leukocytes were used and tests with serum of normal rabbits were made for control. The sera were diluted with salt solution in order to determine at what point the induced phagocytosis would exceed the spontaneous. Typical strains of poliomyelitic cocci were used. Cultures of Streptococcus pyogenes, of 4 different strains of Streptococcus viridans, and of a pneumococcus strain were also studied. The suspensions were made from 24-hour blood-agar-slant cultures, each suspension containing approximately the same number of organisms. The mixtures were incubated at 37 C. for 30 minutes, and the number of cocci taken up by 50 polymorphonuclear leukocytes determined. The serum was heated for 30 minutes at 56 C. before the tests were made.

In all instances in which complement fixation was marked, there was a high content of opsonin, the serum sometimes being active in dilutions as high as 1 : 7680. Moreover, these antibodies were specific for the poliomyelitis cocci, no change in reaction being obtained with the control bacteria. The results correspond to the previous observations on the specific opsonic reaction in the serum of persons recovering from poliomyelitis.

AGGLUTININS

Unheated serum was used in varying dilutions with the serum of normal rabbits for control. The various strains of poliomyelitic cocci were grown on blood agar slants for 24-48 hours and suspended in enough sterile normal salt solution to make a faintly cloudy suspension. Equal amounts of this suspension and the various dilutions of serum were mixed, incubated for two hours at 37 C., and placed in a refrigerator for 24 hours, after which time the final readings were made. Cultures of a hemolytic streptococcus, a pneumococcus, and two strains of a Streptococcus viridans were tested also.

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[3] Mathers and Tunnicliff: Jour. Am. Med. Assn., 1916, 67, p. 1935.

The results are of doubtful value because of the tendency of the poliomyelitis cocci to agglutinate spontaneously. It may be said, however, that specific agglutinins developed to a small degree in the serum of the immunized rabbits, not being definitely demonstrable in dilutions above 1:8 as a rule. In most instances the immune serum agglutinated all the poliomyelitic strains but not the control suspensions. In one case only did a serum agglutinate in a dilution as high as 1:128. Occasionally a serum active for one strain would not agglutinate other strains, but in no instance were the cocci agglutinated by the control sera any more than spontaneously.

SUMMARY

In the blood of rabbits injected with various strains of poliomyelitis cocci, specific agglutinins, opsonins and complement fixing bodies could be demonstrated. The complement fixing bodies were demonstrable in high dilutions and were specific for the poliomyelitis group in most instances. Occasionally, however, some fixation occurred with pneumococcus and hemolytic streptococcus antigens, but this was rare. Conversely, the pneumococcus immune serum and the hemolytic streptococcus immune serum gave some fixation with the poliomyelitic antigens; in the instance of the pneumococcus antigen the reaction seemed apparently nonspecific.

The opsonins were demonstrable in high dilutions and were specific —the serum being without opsonic effect on hemolytic streptococci, green-producing streptococci and pneumococci.

The agglutinins were demonstrable in low dilutions only. Spontaneous agglutination of the cocci interferes with agglutination tests.

These results suggest that serologic tests, especially opsonin determination, may prove of value in the diagnosis of acute poliomyelitis, in case the coccus is found to be associated closely with the disease.